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Article Original Research

Biochemical Composition of Pomegranate (*Punica granatum* L.) as a Natural Feed Additive

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Abstract: Antibiotics and microbial resistance are important issues in the poultry industry. The research continues to find alternative natural feed additives to replace antibiotics and have positive effects as growth promoters. This study aimed to provide the biochemical composition of pomegranate (*Punica granatum*) with the object of peels, seeds (including arils), and whole fruit (peels + seeds) using six replicates for each part of the pomegranate. Eighteen pomegranates were analyzed for water, carbohydrates, protein, fat, fiber, ash, polyphenols, flavonoids, tannins, and antioxidant activity. The weight of the peel compared to the total weight was significantly higher ($p \leq 0.05$) than seeds and whole fruit. The protein, fat, and fiber levels of peels were substantially lower than those of seeds and whole fruit, in contrast, the ash content and carbohydrate levels were significantly higher ($p \leq 0.05$) than other pomegranate parts. The biochemicals and bioactive compounds in the peel tend to be higher than the different parts. The levels of polyphenols, flavonoids, tannins, and antioxidant activity in the peel were significantly ($p \leq 0.05$) higher than all parts of the pomegranate, respectively 11.3 mg/g, 9.78 mg/g, 200.52 mg/g, and 39.03%. This study concludes that the peel is the best contributor as a natural feed additive.

Keywords: biochemical; peels, feed additive, natural, pomegranate

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1. Introduction

The addition of antibiotics as growth promoters in poultry feed leads to resistance of bacterial strains and accumulation of antibiotic residues in meat and eggs. Anti-microbial resistance is a major threat affecting the poultry industry, and antibiotic residues in livestock products adversely affect human health. Pharmacological research on plants for various diseases has been carried out to obtain natural products as substitutes for antibiotics and synthetic drugs. A new trend in the poultry industry is the addition of natural feed additives, including phytobiotics that do not cause bacterial resistance and residues in livestock products.

Phytobiotics include various plant-derived products such as essential oils, extracts, and herbs that positively affect animal productivity and product quality [1][2]. Phytobiotics are effectively used in the poultry industry as growth promoters, antimicrobials, and immunomodulators.

Pomegranate (*Punica granatum* L.) is one of the plants that contains valuable chemical compounds that have high potential fruit to be explored. The pomegranate is native to India and the Mediterranean region [3][4] and is also cultivated in tropical and sub-tropical countries [5]. The production and consumption of pomegranates continue to grow, global pomegranate production in 2017 was approximately 3.8 million metric tonnes. In 2020, the three main pomegranate-producing countries are China, Iran, and India producing about 4.9 million tonnes, more than 80% of world production with a cultivation area of 455,200 ha [6].

Several studies showed pomegranates have high nutritional value and positive effects on improving health, which is getting increasing attention for its health-promoting effects [7]. Many previous studies reported that pomegranate has high antioxidant activity, antimicrobial, anti-inflammatory, non-cytotoxic, hepatoprotective, and growth-promoting properties {Citation} [11][12][13]. Several bioactive compounds have been isolated from pomegranate. These compounds include tannin and other polyphenolic constituents. Polyphenols are secondary plant metabolites classified into several types, namely phenolic acids, flavonoids, catechins, ellagic acid, anthocyanidins, and hydrolysis tannins. Their structures are composed of at least one aromatic ring with one or more hydroxyl substituents [8]. The main phenolic substances among them are tannins, flavonoids, and phenolic acids. Based on their structural characteristics, tannins can be divided into four main groups, gallotannins, ellagitannins, complex tannins, and condensed tannins [9]. As a kind of ellagitannins, punicalagin is the main constituent of pomegranate peel tannins, and the characteristic substance of pomegranate peels, with much higher content than other fractions [10]. Punicalagin can produce ellagic acid through spontaneous endo-esterification hydrolysis of the hexahydroxybenzoic acid structure. Flavonoids are a class of the low-molecular-weight phenolic compounds [11]. These compounds provide reducing and scavenging free radicals [12] [21]

Pomegranate also contains essential minerals such as potassium, phosphorus, calcium, sodium, manganese, iron, and nitrogen [12]. Pomegranate contains vitamin E (a-tocopherol acetate), which is also an important synthetic antioxidant that coordinates several metabolic processes [13]], promotes growth, and activates immune responses to boost immunity [13] [14].

Each part of pomegranate, such as peel, aril, and seeds, presents a very specific composition, generally presenting anti-oxidant and anti-radical compounds. The high potential of chemical compounds with positive health value encourages research to justify the parts of pomegranate that have a beneficial composition by exploring biochemical compounds and their

composition in various parts of pomegranate. Therefore, the objectives of this study were to provide the biochemical composition of pomegranate with the objects of the powder of peels, seeds, and whole fruit. It is hypothesized that different parts of pomegranate affect the biochemical composition of pomegranate as a natural feed additive.

2. Materials and Methods

The study began by separating the pomegranate into peels, seeds including arils (the aril separated by the pericarp sweet pulp), and whole fruit (peels and seeds) manually removed from pomegranate fruits, dried in the shade, and powdered in a grinding mill according to the separation method in reference [15]. The separated materials were cleaned, dried, and ground into powder.

The treatments were peels, seeds, and whole fruit were repeated six times each. Water content analysis uses sterilized, dried, and weighed containers. The sample was inserted and the container was weighed again. It was then dried in an oven at 105ºC and weighed repeatedly until a constant weight was obtained.

Nutritional analysis included estimation of moisture, fat, ash, fiber, protein, and carbohydrate content, which was determined on a dry basis by using the AOAC Method [16][28]. Fat was estimated using petroleum ether as an extract solvent in a Soxhlet apparatus. Total ash was calculated after weighing the combustion residue at 550ºC for 12 hours. Protein was estimated through the Kjeldahl micro-distillation method. The carbohydrate percentage was calculated by the difference method. The antioxidant activity analysis is according to the reference [17] tannin content, flavonoid, and polyphenol analysis using reference [18].

Data obtained from the results of this study were analyzed with analysis of variance (ANOVA) if the results of the analysis showing significant mean differences were continued by the Duncan Test.

3. Results and Discussion

3.1. Chemical composition of Pomegranate

The weight composition of peel, seed, and whole fruit and the results of chemical composition analysis in the form of water, ash, protein, fat, carbohydrate, and fiber content are listed in Table 1.

The percentage of peel weight was significantly higher ($p \leq 0.05$) than seed and whole (peels and seeds). The percentage of peel weight compared to the total weight was 27.67%. Pomegranate can be divided into peels, seeds, and whole fruit, in which peels take up about 26-30% of the total weight. The results of the analysis showed that the peel had higher ash, fiber content, and carbohydrate content compared to seeds and whole fruit, according to the opinion of [19] the peels had a high ash content compared to other parts of the fruit, seeds contain high levels of fat and protein compared to skin and whole fruit powder. The protein content, fat content,

and fiber content of peels were significantly lower ($p \leq 0.05$), while ash content and carbohydrate content were significantly higher ($p \leq 0.05$) than other parts of pomegranate. According to [21] peel contains high amounts of fiber. The ash content was not significantly different ($p \geq 0.05$) from the edible parts.

Table 1. Weight and chemical composition of different parts of pomegranate

Chemicals	Peels	Seeds	Peels + Seeds	Standard Deviation	Standard Error of Mean
Weight, %	27.69 ^{c}	19.37 ^{a}	21.43 ^{b}	5.76	5.22
Water, %	11.31 ^{a}	12.45 ^{b}	12.04 ^{b}	0.57	0.75
Ash, %	6.18 ^{b}	3.39 ^{a}	5.16 ^{b}	0.51	0.34
Fat, %	1.86 ^{a}	2.25 ^{b}	2.25 ^{b}	0.19	0.01
Protein, %	3.60 ^{a}	4.37 ^{b}	4.29 ^{b}	1.33	0.03
Fiber, %	5.16 ^{b}	3.63 ^{a}	3.68 ^{a}	2.02	0.05
Carbohydrate, %	74.99 ^{b}	72.25 ^{a}	72.54 ^{a}	0.79	1.15

Different superscripts in the same row and column indicate significance differences ($p \leq 0.05$)

Evaluation of bioactive compounds is useful in detecting bioactive principles that may lead to drug discovery and development as well as facilitating quantitative estimation of pharmacologically active chemical compounds. The results of bioactive composition analysis on various parts of the pomegranate are shown in Table 2. The contents of bioactive compounds in peels tend to be higher than in edible parts [20]. The levels of polyphenols, flavonoids, and tannins in peels were significantly ($p \leq 0.05$) higher than all pomegranate parts, 11.3 mg/g; 9.78 mg/g, and 200.52 mg/g, respectively. Peels contain high amounts of polyphenols, vitamins, and minerals [21][18]. Pomegranate peels include a variety of high molecular weight polyphenols, such as flavonoids, hydrolyzable tannins, and minerals [21].

Phytochemical screening in this study proved the presence of phenolic compounds and flavonoids in the peel, seeds, and whole fruit. The presence of bioactive compounds in the three parts of pomegranate is responsible for the therapeutic properties of pomegranate. Flavonoids and phenolic compounds are responsible for the strong antioxidant capacity of pomegranate.

The pharmacological value of medicinal plants is due to the presence of various secondary metabolites such as flavonoids, and phenols. Pomegranate contains alkaloids, carbohydrates, proteins, phenolic compounds, and flavonoids [18]. Numerous bioactive substances in peels contain many health benefits as traditional medicines [21][31] including anti-inflammatory and antioxidant properties as demonstrated by several in vitro and in vivo analyses [20]. To make use of the by-product, the bioactive compounds in pomegranate can be utilized as functional additives. The structure and properties of flavonoids have antioxidant activity. Their antioxidant effects are provided by polyphenol hydroxyl groups that can reduce the content of

free radicals. Besides, the catechol hydroxyl groups in complex and condensed tannins give them the ability to chelate iron and transition metals [14].

Table 2. Bioactive composition of different parts of pomegranate

Bioactive	Peels	Seeds	Peels + Seeds	Standard Deviation	Standard Error of Mean
Polyphenols, mg/g	27.69 ^{c}	19.37 ^{a}	21.43 ^{b}	5.76	5.22
Flavonoids, mg/g	11.31 ^{a}	12.45 ^{b}	12.04 ^{b}	0.57	0.75
Tannins, mg/g	6.18 ^{b}	3.39 ^{a}	5.16 ^{b}	0.51	0.34
Antioxidant activity, mg/g	1.86 ^{a}	2.25 ^{b}	2.25 ^{b}	0.19	0.01

Different superscripts in the same row and column indicate significance differences ($p \leq 0.05$)

According to [22] pomegranate peels showed higher antioxidant activity, so it can be concluded that pomegranate peels are a source of fiber with good physicochemical and functional properties. The polyphenol content of peels in this study is 11.30 mg/g, which is close to the results of the research [23] on polyphenol content in peels with different solvent extraction methods of 18 mg/g. Pomegranate peels contain high tannin. The tannin and flavonoid levels in this study were 200.52 mg/g and 9.78 mg/g, respectively. The tannin levels were in the range of reported [24] 193 to 420 mg/g, while the flavonoid levels in this study were below those reported in the range of 84 to 134 mg/g.

In addition to polyphenols and dietary fiber, alkaloids, vitamins, and various mineral elements are also distributed in pomegranate peels. Two Frontiers in pomegranate peel containing acetyl grenadine, 2-(2-hydroxypropyl)-10-piperidine, sedridine, and N-acetyl serine that belong to alkaloids. In addition, pseudo grenadine, N-methyl grenadine, and iso-grenadine were also found in pomegranate peel [25]. Pomegranate peels also contain alkaloids, vitamins, steroids, and various minerals compared to edible parts, including K, P, Na, Ca, Mg, and N, which play an important role in maintaining normal physiological functions of the body[19].

The complex bioactive compounds in pomegranate peel provide synergistic effects to produce physiological functions [39]. Antioxidant activity is highest in pomegranate peel, so it is a source of antioxidants as well as anti-bacterial which is associated with high phenolic compounds that have physiological effects to prevent and treat infections.

Phenolic compounds play a role in inhibiting enzyme activity causing bacterial death [26] as well as stimulating inhibition of Escherichia coli in the small intestine [27]. Other studies proved the inhibition of Gram-positive bacteria. *Staphylococcus aureus*, and *Staphylococcus epidermidis* [42] Listeria monocytogenes, Bacillus cereus, and Salmonella [28]. Tannins may also exert antibacterial activity through the following mechanisms: inhibition of enzyme activity, precipitation of membrane proteins, and depletion of metal ions [25].

4. Conclusions

Pomegranate peel contains a high percentage of dry matter, carbohydrates, and fiber compared to seed and whole fruit, as well as a high proportion of bioactive polyphenols, tannins, and flavonoids that have antioxidant, antibacterial, anti-inflammatory, and reduced free radicals, so the peel has functional activities that can be applied to animal feed to improve animal health and feed efficiency. Pomegranate peel contains bioactive compounds as a natural feed additive.

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